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THE EFFECT OF MONOCHROMATIC ULTRA-VIOLET LIGHT OF MEASURED INTENSITIES ON BEHAVIOR OF PLANT CELLS[1]

PRELIMINARY REPORT

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INTRODUCTION AND DISCUSSION OF PREVIOUS WORK

The effect of ultra-violet radiation on plants has been the subject of numerous investigations, but most of the results obtained are either indefinite or contradictory. Hardly anything is yet known about the physical and chemical reactions involved. To make any progress in this direction, that is, to seek a physical and chemical explanation for the action of ultra-violet radiation on living cells, it would be essential to accumulate exact quantitative data on the subject. The lack of quantitative measurements of the spectral qualities of the source of radiation is notably the weakest point in most of the investigations, and therefore it becomes almost impossible to correlate or interpret the results obtained.

According to the fundamental law of photochemistry (Grotthuss-Draper Photochemical Absorption Law) only rays which are absorbed are effective in producing chemical changes. However, not all absorbed energy has to result in chemical reactions,

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since radiation may be transformed into another form of radiant energy or produce a change in the energy content of the molecule. According to Einstein's concept of quantum absorption, the occurrence of photochemical reactions is due to the absorption of quanta of radiation, each single molecule requiring one quantum of a frequency characteristic of the absorbing molecule. This concept, based on parallelism with the photoelectric effect, indicates a more or less specific action of different quanta. A quantum of a given energy value can be expressed as a particular frequency or wave length. Of course, one should not forget that the absorption of radiant energy by a molecule is much more complex than it is in an atom and that Einstein's concept applies only to the primary stage of a photochemical reaction. Secondary chemical processes initiated by the primary stage will often be entirely independent from the light action.

It is clear, however, that the use of monochromatic light will help to explain many of the essential factors in photochemical and photobiological investigations.

The necessity of confining one's self to the study of the action of narrow spectral regions in the ultra-violet becomes especially clear if one considers the nature of absorption bands in this part of the spectrum. Ribaud ('13), who studied and compared absorption bands of different gases, liquids, and solids in different spectral regions, came to the conclusion that the width of the absorption bands decreases continuously on going from the infra-red toward the ultra-violet end of the spectrum and is nearly proportional to the wave-length maximum. It is also clear that as long as the absorption of radiant energy involves a given quantum per absorbing molecule the number of quanta or the intensity of absorbed radiant energy must be determined. The Bunsen-Roscoe reciprocity law of photochemistry states that when the product of intensity and exposure time is constant a constant photochemical reaction results. With some modifications the law holds for most of the chemical compounds tested.

As far as the biological action of radiant energy is concerned, there is no reason to believe that the laws of photochemistry are not applicable, even though the situation be much more

complex. As a matter of fact, several of the photochemical laws have been tested for biological objects. The correlation between the absorption spectrum of chlorophyll and the rate at which carbon dioxide is decomposed by the plant was studied as early as 1875 by Timiriazev ('75). Gates ('30), using accurate quantitative measurements, found that the absorption curve of bacteria corresponded strikingly to the curve obtained for the lethal action of different wave lengths and intensities of the incident energy.

Verhoeff and Bell ('16), in their investigations on the harmful effect of ultra-violet radiation on the cornea of the eye, found that the time of exposure necessary to produce symptoms of injury is inversely proportional to the intensity of radiation of the effective ray. Similar results were obtained by Hill and Eidenow ('23) and Weinstein ('30) with paramecia, and Barr and Bovie ('23) with amoebae. Coblentz and Fulton ('24) emphasized the fact that longer exposures do not fully compensate for decreased intensity. An intensity reduction to 1/50 required an increase of $\times 75$ in the exposure time to produce a comparable reaction on bacteria. Gates ('29a) tested the validity of this Bunsen-Roscoe law by the killing effect of ultra-violet radiation on bacteria. He worked with monochromatic light and, measuring intensities by means of a sensitive thermopile, found that the law does not hold strictly, especially with young and metabolically and genetically active bacteria, although it is fairly accurate if small differences in intensities are used.

The results obtained by Verhoeff and Bell ('16), Bovie ('16), Barr and Bovie ('23), and Weinstein ('30) indicate that within certain limits, the same total exposure is required to produce the effect when the radiation is interrupted for short intervals as when it is continuous.

In the light of the preceding discussion it becomes evident that the quality and quantity of light play important parts in the effect it will produce on living matter.

The literature on the action of ultra-violet radiation on plants has been reviewed by Eltinge ('28), Arthur and Newell ('29), and Fuller ('31). As emphasized by Fuller, the fact that sources of radiation of unknown spectral quality have been used in

most investigations makes it almost impossible to compare the results obtained by various workers. He also emphasized the fact that in biological effects radiation from artificial sources of light, such as mercury vapor arcs, can by no means be compared with the radiation of the sun.

To eliminate some of the uncertainty about the spectral aspects of the source of radiation, a number of workers have used filters of various makes to limit the radiation to certain parts of the spectrum. However, unless spectrographs of the transmission of light through the filter are given one cannot be certain about the quality of the spectrum, since commercial mercury vapor arcs vary in this respect according to make, the length of time they have been used, etc. Furthermore, the use of selective filters introduces a number of complications due to the partial absorption of spectral lines other than those eliminated. The relative intensities of the different parts of the spectrum are thus distorted, and one does not know whether the effect produced by interposing a filter is due to the elimination of a certain spectral region or to the weakening of the intensity of the wave lengths transmitted. Besides, spectrographs are usually taken by interposing the filter between the spectroscope and the source of light placed close to the filter, while the objects during irradiation are placed at quite a distance from the source, sometimes as much as 100 inches. Henri (cited by Taylor, '31) claimed to have shown that a strict relationship exists between the infra-red and ultra-violet in their photochemical action. Reiter and Garbor ('28) claimed to have established an antagonistic relationship between the action of two different bands in the ultra-violet spectrum on cell division.

The only way then to get a clear picture of the action of ultra-violet light on organisms would be to use monochromatic light of measured intensities, so that the actual energy falling on the object under investigation could be definitely determined. It is true that monochromatic light does not occur in the natural surroundings of the plant, and therefore cannot be regarded as a normal environmental factor. However, the selective absorption of light by the organic substances of the plant and the fact that it affords the only accurate means of determining the

quantity and quality of light make it advisable to investigate, first, the biological action of monochromatic light and, later on, to synthesize the results.

Several investigators have exposed their objects to radiation passed through a quartz spectrograph. Ward ('93) was the first to use this method for the study of the bactericidal action of light. He was followed by a number of workers.

Hertel ('05) seems to be the first who fully recognized the importance of quantitative measurements of the intensities of monochromatic light used for biological studies. Using a quartz prism and lenses, he constructed a monochromator similar to those used in ultra-violet microscopy. He determined the relative intensities of the lines by means of a thermopile and varied the intensity by regulating the amperage of the metallic arc. He used four lines of the ultra-violet part of the spectrum and studied their effects on paramecia, diatoms, *Oscillaria*, and *Elodea*. He found that the line 2800 Å was the most powerful in its destructive action on cells, and noticed that not only was the streaming in the cells of *Elodea* retarded by the light but also that the cells finally died.

Schulze ('09) devoted himself to the study of the effect of the powerful line of 2800 Å of the magnesium spark. As objects he used cells of *Spirogyra*, *Nitella*, *Vallisneria*, and *Elodea*, root hairs of *Hydrocharis*, anther hairs of *Tradescantia*, and hyphae of *Mucor*. He employed a monochromator similar to that used by Hertel and focused the rays by means of quartz lenses on the stage of the microscope. The intensity was varied by means of regulating the amperage across a magnesium spark. He found that at certain intensities small vacuoles appear in the cells, that protoplasmic streaming is retarded, and that longer exposures result in death of the cells. The growth of hyphae of *Mucor* and the cell division in *Tradescantia* were retarded. Even when using relatively small intensities he was unable to detect stimulation. By means of microphotographs he showed that the cuticle and epidermis strongly absorb the ultra-violet of this frequency. Parenchyma tissue, phloem, and young cambium were quite transparent to the light, whereas xylem again absorbed it rather strongly. As far as the different parts of the cell were

concerned, he showed that the strongest absorption was in the middle lamella. Strong absorption was also shown by the nuclei and chromosomes. Unfortunately it is impossible to ascertain the exact intensities used in his experiments.

Frank and Gurwitsch ('27), in trying to discover the cause of the so-called mitogenetic radiation which they claimed is emitted by embryonic tissue, used a small quartz spectrograph to determine the physical nature of the radiation. They believed that the wave lengths of 1930–2370 Å at one-minute exposure produced a stimulating effect on the cell division of the root of the onion similar to that produced by mitogenetic rays.

Reiter and Garbor ('28), in their extensive study on mitogenetic rays, employed a specially constructed spectrograph which permitted them to combine at one focal point several wave lengths. By using a number of arcs and sparks to obtain a large number of lines in the ultra-violet part of the spectrum, they found that the line 3400 Å, and to a lesser degree, line 2800 Å produce a stimulating effect on cell division in the root of the onion, eggs and larvae of the frog and salamander, and sarcoma tissue of the rat. This was evident only at relatively low intensities, whereas at higher intensities the same rays were destructive. If the spectral region of 2900–3200 Å was added to the radiation of the line 3400 Å, the stimulating as well as destructive action of the radiation disappeared. Frank ('29) disagreed with Reiter and Garbor as to the wave length involved in mitogenetic radiation. In rechecking his earlier observations he found that the spectral region between 2000 and 2400 Å is effective. He even claims to have obtained a mitogenetic stimulation of yeast in this region by passing the radiation from that of a biological source through a powerful quartz spectrograph. Neither Reiter and Garbor nor Frank gave the measurements for the intensities employed.

Recently Gates ('29a, '29b, '30) published a series of investigations on the bactericidal action of ultra-violet light. Using monochromatic light from a specially constructed powerful monochromator and measuring the intensity of the incident radiation in absolute energy units by means of a thermopile, he studied the lethal effects of ten lines of the mercury vapor

spectrum on *Bacillus coli*, *B. communis*, and *Staphylococcus aureus*. These papers have probably contributed more to the clarifying of the topic under discussion than all the rest of the investigations taken together and demonstrated the value of the use of monochromatic light of measured intensities in the study of biological objects. In the first paper of the series Gates determined the curves of bactericidal action for each wave length studied. He found that with all the different wave lengths the reactions followed similar curves, but occurred, at each wave length, at a different energy level. In his second paper he studied the effect of various environmental factors on the bactericidal action of ultra-violet and determined the wave length limits of the action as being between 3130 and 2250 Å, although the lower limit could not be definitely ascertained. In the third paper the absorption curves of the body of the bacteria were determined and correlated with the curve of incident energy instrumental in the bactericidal action. Although some minor differences are evident in the curves, they form in general a reciprocal of each other. He proved that the belief that the shorter the wave-length the greater the bactericidal action of ultra-violet is erroneous, and that a striking maximum of effectiveness exists between 2600 and 2700 Å. Having thus accumulated quantitative data on the subject, he promises a discussion of the photochemical action of the radiation in his next paper.

Weinstein ('30), also using similar quantitative measurements and focusing monochromatic light on the stage of a microscope, made observations of the effect of five wave lengths of the ultra-violet part of the spectrum on *Paramecium micromultinucleatum*. The line of 2650 Å was found to be most effective in killing paramecia.

Marshall and Knudson ('30), by means of similar methods, studied the effect of monochromatic light on the formation of vitamin D from ergosterol. They found that the rate of production of the vitamin is directly proportional to the number of light quanta absorbed by ergosterol. The reaction was found to be independent of the wave length within the absorption region of ergosterol.

Apparatus Used

To be able to obtain quantitative data on the action of ultraviolet radiation on plant cells a Bausch and Lomb quartz monochromator was used. The instrument is of the constant deviation type and calibrated for the wave lengths from 2000 to 8000 Å. The intensity of the radiation was measured by means of a Coblentz bismuth-silver thermopile adapted by the B. & L. Optical Company for their monochromator. The thermopile is mounted on an adapter in such a manner that it can be lowered in front of the slit when intensity measurements are taken and raised above the slit to permit the light to pass into the space. The relative positions of the thermopile (b) in its adapter (c) can be seen by comparing figs. 1 and 3, pl. 42. The metal box containing the thermopile and supplied with a quartz window was sealed air-tight and evacuated through an outlet (d). It was found that, although it was difficult to maintain a high vacuum for long periods, one could get a relatively constant partial vacuum by evacuating the box while measurements were taken. An oil vacuum pump was used for evacuation.

The thermopile was connected with a Type HS Leeds and Northrup galvanometer having a sensitivity of 20 mm. per μv. and an internal resistance of 16.8 ohms. Since the resistance of the thermopile was found to be equal to the critical damping resistance of the galvanometer (7.5 ohms) no additional resistances were placed in the circuit. The deflections were read through a telescope at 2 meters scale distance.

The thermopile was calibrated in absolute energy units (mechanical equivalent) against a Bureau of Standards carbon filament incandescent lamp No. C-109. The reaction of the thermopile was found to be linear, and a 1 mm. deflection on the galvanometer corresponded to a light energy of 7.22 erg/mm.^{2}/second. The readings of the intensity of the different spectral lines taken at various times were quite constant, varying within the limit of 5 per cent.

As a source of light, at first a horizontal air-cooled quartz mercury vapor arc of the Burdick Cabinet Company was tried but proved to be of insufficient intensity for monochromatic work. A vertical water-cooled Burdick arc (type W-910) operated

at 55 volts and 4.3 amperes was used in most of the experiments. It was placed at a distance of 4 cm. from the entrance slit of the monochromator. This lamp is supplied with a filter of water 1 cm. deep, so that most of the infra-red radiation is probably eliminated.

The distribution of the spectral lines in the ultra-violet region and their relative intensities as measured by the thermopile are represented in fig. 1.

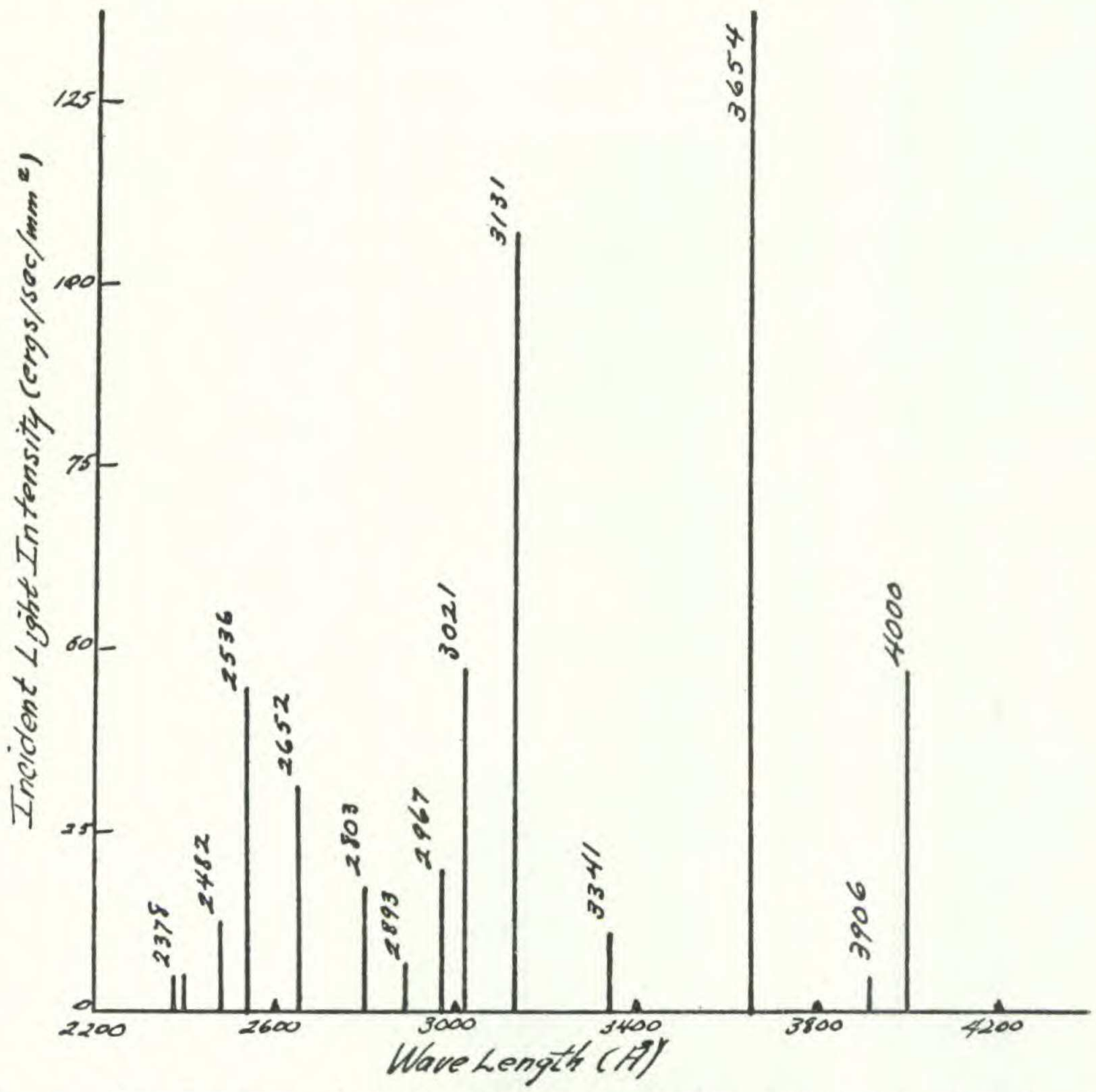

Fig. 1. Distribution and relative intensities of the spectral lines of a Burdick water-cooled mercury arc.

The widths of the entrance and exit slits of the monochromator were kept constant at 0.2 mm., representing an area of 2 mm.^{2}

Considerable difficulties were experienced in so adjusting the objects studied that they could be examined by a microscope and the incident energy could be determined by the thermopile. Even at a distance of 1 cm. from the exit slit of the monochro-

mator the rays are dispersed to a considerable extent. As illustrated in pl. 42, figs. 1 and 2, a metallic tube (e) supported by an adjustable stand (g) was placed in the path of the ray passing from the monochromator (j). By means of a quartz lens (f) the image of the exit slit of the monochromator was reproduced on a second slit just behind the thermopile (b). One end of the tube was shaped in such a manner that the whole thermopile adapter (c), including the thermopile and the adjustable slit, could be removed during the raying and slipped into position during intensity measurements.

Plate 42, fig. 2, shows the set-up during the raying. A vertical adjustable microscope stage (h) supporting a slide with the object (i) under investigation was placed in front of the tube. In this way the image of the exit slit of the monochromator was focused on the subject. The observations were made, and the focusing of the ray was done by means of a horizontal microscope (a). For microscopic examination of the objects during the experiments a micro-lamp was interposed between the lens (f) and the monochromator (j). Since the quartz lens absorbed a large percentage of the radiation of wave lengths below 2536 Å, some of the experiments were carried out in a set-up as illustrated in pl. 42, fig. 3, and referred to in the tables as position B. By means of a projection built on the microscope stage the objects were brought into a position directly in front of the exit slit of the monochromator. With this arrangement, however, it was impossible to make careful examination of the objects during the experiments.

Table 1 gives the intensities for the two positions A and B as illustrated in pl. 42, figs. 2 and 3. The total intensities of the light falling on the object at a slit width of 0.2 mm. or per 2 mm.^{2} area are given for each spectral line.

SELECTION OF OBJECTS AND PROCEDURE

The selection of objects was limited, due to the necessity of having them exposed to a small area of light (2 mm.^{2}). Since ultra-violet radiation does not penetrate deeply into plant tissue and is known to be absorbed readily by single layers of cells, objects of single-cell thickness had to be selected. It was also im-

TABLE I

INTENSITIES OF LIGHT PER 2 SQ. MM. IN POSITIONS A AND B

Wave lengths	Intensities for position A (pl. 42, figs. 1–2)		Intensities for position B (pl. 42, fig. 3)	
Å	galv. defl. mm.	ergs/sec/2 mm.²	galv. defl. mm.	ergs/sec/2 mm.²
3663.27, 3662.87, 3654.83, 3650.15	24.5	196.0	36.0	280.0
3341.48	1.5	12.0	2.8	22.4
3131.84, 3131.56, 3125.60	10.5	84.0	26.8	214.4
3021.50	5.0	40.0	11.7	93.6
2967.28	2.7	21.6	5.1	40.8
2893.60	0.9	7.2	2.0	16.0
2803.50	2.0	16.0	4.3	34.4
2652.00	3.0	24.0	7.7	61.6
2536.00, 2534.80	2.0	16.0	11.2	89.6
2482.70	0.5	4.0	3.0	24.0
2378.30	0.3	2.4	1.0	8.0

portant that the objects should be transparent to visible light so that their cell contents could be examined under the microscope. Several objects were tried but few of them proved satisfactory for the purpose.

To determine their suitability various objects were placed at a distance of 10 cm. from the open water-cooled mercury vapor arc and their reaction to the radiation was observed. The cells of *Chlamydomonas* were found to be very sensitive to the action of the light from the arc. After exposure of 10 minutes their motion ceased, and in about 20 minutes a complete destruction of the cell contents was observed. Due to the motility and small size of the cells it was impossible to confine them within the narrow area (2 mm.²) of irradiation. Although the cells of the filaments of *Spirogyra* were markedly injured by the radiation of the arc after 30-minute exposures, it was difficult to obtain conclusive results, since even non-radiated cells showed a large variation in their response to plasmolyzing agents. *Cladophora* and *Pleurococcus* did not show any marked changes in their cell structure after exposures to the arc for ½ hour. Strips of the epidermis of *Rhoeo discolor* lost their purple pigment after an

exposure to the arc of about 1 hour, but due to their overlapping the vitality of the cells was difficult to judge.

The objects selected as most suitable were leaves of the gametophyte of a common local species of *Mnium* and stamen hairs of *Tradescantia reflexa* Raf. Single leaves of *Mnium* were carefully detached from the stem under the dissecting microscope. Six to nine leaves were then placed in a drop of tap water on a large glass cover-slip 43 × 50 mm. serving as a slide, and covered with a quartz cover-slip. This made it possible to observe the objects with a microscope from both sides. To prevent any pressure on the object, glass capillaries were interposed between the two cover-glasses. The object (i, pl. 42, fig. 2) after a microscopic examination was placed on a vertical adjustable microscope stage (h, pl. 42, figs. 2 and 3) provided with an opening in the center. Three of the leaves were then moved to such a position that the radiation, passing through the slit (2 mm.^{2}) of the monochromator, was focused on them. Thus an area of approximately 0.3 mm.^{2} of each leaf was exposed to the irradiation. The other leaves served as controls.

A similar procedure was adopted for the stamen hairs of *Tradescantia*. They were removed from the filament by means of a pair of pointed scissors, examined to detect possible injury, and then irradiated. At least three hairs were placed in such a position that they were in the path of the light. In each experiment at least eight cells were irradiated.

The exposure of the leaves of the moss plant to the full arc at a distance of 10 cm., for at least 4 hours, produced a decolorization of the chloroplasts. In extreme cases all the chloroplasts were completely deprived of the green color, but transitional stages could be observed. The cell walls appeared as if stained green by the diffused chlorophyll. In the case of one leaf overlapping another, the part shielded had green chloroplasts, although the effect of irradiation was indicated by the green color of the cell walls.

There was no visible change in the starch content of the chloroplast, but in the case of completely decolorized cells no plasmolysis could be produced, indicating destruction of the protoplasmic membrane. A similar effect was obtained by

exposing the leaves for 12 hours to a 1000-Watt Mazda lamp at a distance of 40 cm. However, to produce an effect comparable to that of the arc, an exposure of 12 hours was necessary. After the experiment the irradiated and control leaves were examined. Two of the irradiated leaves were plasmolyzed by an 8 per cent solution of KNO_3, and the third one was placed in a watch-glass with tap water for 3 to 8 hours to observe any possible after-effects of the irradiation. Cells of detached moss leaves used as controls did not show any detectable injury and responded readily to plasmolyzing if left in water for 24 hours.

The *Tradescantia* hairs had purple, vacuolar contents, so that the protoplasmic streaming was readily discernible. Unradiated hairs kept on the stage of the microscope for more than 24 hours showed no visible change in protoplasmic streaming, provided they were handled carefully and not subjected to pressure of the cover-glass. On exposure to the open mercury arc (at a distance of 10 cm.) the streaming ceased in 20 minutes and coagulation of the protoplasm was evident.

All the experiments with monochromatic light on the moss plant were carried out with the object and stage in position B, that is, directly in front of the exit slit of the monochromator (pl. 42, fig. 3). The experiments with the stamen hairs were carried out in position A (pl. 42, fig. 2) for the longer wave lengths and in position B (pl. 42, fig. 3) for the shorter wave lengths. As can be seen from table 1, the intensities at position B were greater than at position A, and were therefore preferred for the moss leaf which was easily centered on the image of the slit by the low power of the microscope. In this position it was not possible to observe the behavior of the cells during irradiation, and the effect was determined by examining the exposed area at the close of the experiments. Position A was preferred for the experiments with stamen hairs, since by interposing a micro-lamp between monochromator and focusing lens (f and j, pl. 42, fig. 2) examinations with 16-mm., 8-mm., and 4-mm. objectives could be made. In the first experiment, with every spectral line tested, microscopic examinations were made at 10- to 15-minute intervals. In the succeeding ones, no examinations were made up to the close of the experiment. In this way a possible

effect of the micro-lamp was eliminated. The adjustment of the hairs over the slit in position B was somewhat difficult because the position of separate cells could not be accurately ascertained. The position of whole hairs, however, was easily determined.

It was of course impossible to test all of the lines of the mercury vapor spectrum or separate the closely adjacent ones. As can be seen from table I, eleven lines (or groups of lines) had been selected. All of them were easily separable with the slits at 0.2 mm. Two to three experiments were conducted for each of the lines used. The time of exposure was in most cases two hours, although in a few instances it was extended to as much as four hours. Longer exposures were not used due to technical limitations.

EXPERIMENTAL RESULTS AND DISCUSSION

The results obtained are represented in tables II and III. No visible distortions in the cell structure were observed with any of the wave lengths or intensities used. All of the irradiated leaves of *Mnium* had a normal appearance. No distortions in the protoplast were visible, and the cells plasmolyzed readily if placed in an 8 per cent solution of KNO_3. No after-effects of the radiation were noticed. It might be possible that a slight discoloration of the chloroplast took place, but if so it was not clearly detectable. In one experiment after an intermittent exposure of eight hours (129.02×10^4 ergs/mm.^{2}) a discoloration comparable to that produced by the open mercury arc was produced. Since this is a single instance and the experiment has not been repeated, it is not taken into consideration in this paper.

In the case of the stamen hairs of *Tradescantia*, protoplasmic streaming was observed in all the cells irradiated. No visible distortion in the cell content was noticed. It seemed that in the case of line 2893 Å the protoplasmic streaming was retarded. The rate of streaming of the protoplasm, however, varied considerably in different cells, hence there is some difficulty in using it as a criterion of vitality.

There is no doubt that stronger intensities, at least for some ultra-violet lines, would produce a killing effect and destruction of the protoplast in plant cells.

From the data available for the energies of ultra-violet monochromatic light required to produce the killing of bacteria and

TABLE II

CORRELATION OF WAVE LENGTH, INTENSITY, TIME OF EXPOSURE, AND INCIDENT LIGHT ENERGY PER SQ. MM., USED IN THE EXPERIMENTS WITH LEAVES OF MNIUM

Wave lengths Å	Incident light intensity ergs/sec/mm.^{2}	Experiment No.	Time of exposure hours	Total incident light energy ergs/mm.^{2} $\times 10^4$
3654	140.0	I	2	100.80
		II	2	100.80
		III	2	100.80
3341	11.2	I	2	8.06
		II	2	8.06
		III	3	12.10
3131	107.2	I	2	77.18
		II	2	77.18
		III	2	77.18
3021	46.8	I	2	33.70
		II	2	33.70
2967	20.4	I	2	14.69
		II	2	14.69
2893	8.0	I	2	5.76
		II	2	5.76
		III	4	11.52
2803	17.2	I	2	12.38
		II	2	12.38
		III	2	12.38
2652	30.8	I	2	22.18
		II	2	22.18
2536	44.8	I	2	32.26
		II	2	32.26
		III	3	48.38
2482	12.0	I	2	8.64
		II	2	8.64
		III	2	8.64
2378	4.0	I	2	2.88
		II	2	2.88
		III	4	5.76

Constants: Total area irradiated = 2 mm.^{2} Approximate area of each leaf exposed to irradiation = 0.3 mm.^{2} Microscope stage and object in position B (pl. 42, fig. 3).

Effect: No visible changes in cell structure. Normal plasmolysis with 8 per cent KNO_3.

TABLE III

CORRELATION OF WAVE LENGTH, INTENSITY, TIME OF EXPOSURE, AND INCIDENT LIGHT ENERGY PER SQ. MM., USED IN THE EXPERIMENTS WITH STAMEN HAIRS OF TRADESCANTIA

Wave lengths	Incident light intensity	Experiment No.	Time of exposure	Total incident light energy
Å	ergs/sec/mm.^{2}		hours	ergs/mm.^{2} $\times 10^4$
		I*	2	70.56
3654	98.0	II*	2	70.56
		III*	1	35.28
		I*	2	4.32
3341	6.0	II*	2	4.32
		I*	2	30.24
3131	42.0	II*	2	30.24
		III*	2	30.24
		I*	2	14.40
3021	20.0	II*	2	14.40
		III*	2	14.40
		I*	2	7.78
2967	10.8	II*	2	7.78
	3.6	I*	1	1.18
2893	8.0	II	3	8.64
		I*	2	5.76
2803	8.0	II*	2	5.76
		III*	1	2.88
	12.0	I*	1	4.32
2652	30.8	II	2	22.18
	8.0	I*	2	5.76
2536	44.8	II	2	32.26
	44.8	III	3	48.38
		I	1	4.32
2482	12.0	II	2	8.64
		I	2	2.88
2378	4.0	II	3	4.32

Constants: Total area irradiated = 2 mm.^{2} Approximate number of cells irradiated = 8.

Effect: No visible injury to the cell. Protoplasmic streaming continued.

In experiments indicated by asterisk (*) microscope and object were in position A (pl. 42, fig. 2); in the others, in position B (pl. 42, fig. 3).

paramecia (Gates, '29, Weinstein, '30), it was anticipated that the comparatively strong energies used in the present investigation would be sufficient to produce a similar effect on plant cells. This is apparently not the case.

TABLE IV

COMPARISON OF TOTAL ENERGIES (EXPOSURE TIME × INTENSITY) USED IN THIS INVESTIGATION WITH THOSE USED BY GATES AND WEINSTEIN

Wave length Å	Ergs/mm.²			
	Used in present investigation		Gates*	Weinstein
	Tradescantia	*Mnium*	necessary to kill 100% of bacteria	necessary to kill paramecia
3654	705,600	1,008,000		
3341	43,200	80,600		
3131	302,400	771,800		19,629
3021	144,000	337,000	13,000	10,850
2967	77,800	146,900	3,000	
2893	25,900	57,600	725	
2803	57,600	123,800	475	2,473
2652	86,400	221,800	350	2,162
2536	322,600	322,600	325	2,284
2482	86,400	86,400	350	
2378	28,800	28,800	540	

* Calculated from curves Gates ('29), p. 240.

TABLE V

COMPARISON OF INTENSITIES (ENERGY PER SECOND) USED IN THIS INVESTIGATION WITH THOSE USED BY GATES AND WEINSTEIN

Wave length Å	Ergs/mm.²/sec.			
	Intensities used in this investigation		Intensities used by Gates	Intensities used by Weinstein
	Tradescantia	*Mnium*	Bacteria	Paramecia
3654	98.0	140.0		
3341	6.0	11.0		
3131	42.0	107.2		7.27
3021	20.0	46.8		5.48
2967	10.8	20.4		
2893	8.0	8.0		
2803	8.0	17.2		1.33
2652	30.8	30.8	11.0	2.72
2536	44.8	44.8		1.12
2482	12.0	12.0		
2378	4.0	4.0		

If we compare the total energies (time of exposure × incident intensity) of this investigation with those used by the two

authors (table IV), it is apparent that much larger energies were used in this experiment.

Although the Roscoe-Bunsen photochemical reciprocity law has been shown to be fairly accurate for some biological objects, there still might be some doubt as to its applicability for exposures as long as two hours. Allowance in this case must be made for a possible concurrent recovery process of the organism during irradiation. However, table V shows that the intensities used in this experiment were larger by a factor of 3 to 40, and therefore the greater energies were due to increase both of intensity and time of exposure. It is therefore evident that the plant cells used are much more resistant to the lethal action of ultra-violet than bacteria and paramecia.

Several attempts have been made to explain the lethal action of ultra-violet radiation. Its killing action is usually ascribed to the destruction or precipitation of some of the constituents of the protoplasm. Henri ('12) found that the abiotic power of ultra-violet rays is proportional to the coefficient of absorption of the protoplasm. Gates ('28) believes that the destruction of certain nucleoproteins is responsible for the killing of the cell. However, it is clear that the resistance of a cell to the action of the rays will depend also on its structural characteristics, such as size of the cell, nature of its cell wall, presence or absence of pigments, etc. Henri ('12) showed that in small cells the entire protoplasm was affected by the rays, whereas in larger cells only a surface reaction resulted. Schulze ('09) noticed that the cell wall and especially the middle lamella are the parts of the cell which absorb most of the radiation if exposed to line 2800 Å. The comparatively high resistance of our objects to the lethal action of ultra-violet radiation might then probably be explained by the characteristic structure of the cells of higher plants.

SUMMARY

1. The necessity of quantitative data for the study of the effect of ultra-violet light on plants is discussed.

2. An experimental arrangement for securing monochromatic light of measured intensities and its application for use with plant objects are described.

3. Leaves of *Mnium* and stamen hairs of *Tradescantia* are exposed to the radiation of eleven lines of the mercury spectrum.

4. It has been shown that no visible injury to the cells resulted from exposure to relatively strong intensities.

5. By comparing these results with those obtained by other workers it has been shown that the plant cells used are very much more resistant to the lethal action of ultra-violet light than bacteria and paramecia.

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Explanation of Plate

PLATE 42

Fig. 1. Position of monochromator, focusing device, and thermopile during intensity measurement (A).

Fig. 2. Position of monochromator, focusing device, and stage supporting object during irradiation (A).

Fig. 3. Microscopic stage and object in position B close to exit slide of monochromator.